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10/528,833	06/16/2006	David Durantel	P08599US00/BAS	3759

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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/528,833	DURANTEL ET AL.	
	Examiner	Art Unit	
	SAMUEL WOOLWINE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 December 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-28 and 31-78* is/are pending in the application.

4a) Of the above claim(s) *31-36* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-15,17-28,37-51,53-58 and 60-78* is/are rejected.

7)☒ Claim(s) *9,16,52 and 59* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *22 December 2008* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status

Applicant's reply filed 12/22/2008 is acknowledged. Claims 1-28 and 31-78 are pending in the application (claims 37-78 are new). Claims 31-36 remain withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election made in the reply filed 06/03/2008 has been treated as an election without traverse (MPEP § 818.03(a)).

The rejections made under 35 U.S.C. 112, 2nd paragraph in the Office action mailed 08/20/2008 are withdrawn in view of Applicant's amendment.

The rejection of claims 4, 17 and 20 under 35 U.S.C. 103(a) made in the Office action mailed 08/20/2008 is withdrawn. After further consideration, the examiner finds the rejection of these claims insufficient to establish *prima facie* obviousness. These claims are subject to new grounds of rejection set forth below.

Any rejection not reiterated below may be considered withdrawn as no longer applicable.

Some of the new grounds of rejection in this Office action were not necessitated by amendment. This action is NON-FINAL.

Sequence Compliance

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. §

1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825 for the reason(s):

37 CFR 1.821(c) requires:

> Each sequence disclosed must appear separately in the "Sequence Listing."

37 CFR 1.821(d) requires:

> Where the description or claims of a patent application discuss a sequence that is set forth in the "Sequence Listing" in accordance with paragraph (c) of this section, reference must be made to the sequence by use of the sequence identifier, preceded by "SEQ ID NO:" in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application.

Figures 2 and 3 disclose nucleotide sequences that are not presented with SEQ ID NOs. If not already present in the Sequence Listing, these sequences should be added to thereto, and should be presented in the figures together with their corresponding SEQ ID NOs.

Drawings

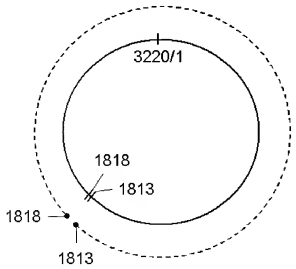
The replacement drawings were received on 12/22/2008. These drawings are not accepted. The text in figures 1, 2 and 4 is still unreadable (either because of shading or, in the case of figure 1, too small to be seen). Furthermore, figures 2 and 3 contain nucleic acids sequences but lack corresponding sequence identifiers as discussed above.

Claim Objections

Claims 9, 16, 52 and 59 are objected to. These claims recite non-elected SEQ ID NOs. These claims are only considered with regard to the elected SEQ ID NOs 1 and 17.

Preliminary Note

The instant application and the prior art discussed in this Office action are directed to the synthesis of "greater-than-genome length" hepatitis B virus (HBV) constructs. The genome of HBV is a circular double-stranded DNA molecule. The instant application, as well as the prior art, will often refer to a fragment as comprising, e.g., "nucleotides 1818-1813" (see claim 3) or "nt 1686-660" (see Schories et al, Journal of Hepatology 33:799-811 (2000), page 802, column 1, "Fragment A"). Where the first number of the range is larger than the second number, this implies that the fragment extends from the first numbered position, through the HBV genome, across the junction of the last and first nucleotides of the genome, to the second numbered position. For example, assuming a genome size of 3220 nt, a fragment designated as 1818-1813 would correspond to the dotted segment below, shown in relation to the circular genome (where position 1818 represents the 5' end of the fragment, and position 1813 represents the 3' end of the fragment):



Note also that numbering of HBV nucleotide positions varies in the prior art; for example, the nucleotide designated as position 1 in one publication may not correspond to the nucleotide designated as position 1 in another publication.

Claim Interpretation

The phrase "transcriptable in pgRNA" (for example, recited in claim 1) will be construed as meaning "able to be transcribed into pgRNA" (pre-genomic RNA); see page 5, paragraph beginning "The present invention...such that a pgRNA can be synthesized from this DNA post-cell-transfection".

The term "incidence" in the phrase "incidence of the pharmaceutical product" (for example, recited in claim 42) will be assumed to mean "effect".

The term "the +1 of transcription" (recited for example in claim 2) will be assumed to mean the nucleotide corresponding to the first nucleotide of the HBV pre-genomic RNA transcript.

The term "the ATG of the pre-C gene" recited in claim 2 will be assume to mean the ATG start codon of the pre-C gene.

The terms "in 5' from" and "in 5' of" recited in claim 2 will be construed as "5' of".

The "about 1 genome unit" recited in claim 2 will be construed as a fragment of the circular HBV genome, wherein the 5' end of the fragment corresponds to "the +1 of transcription" as discussed above, or to a nucleotide 5' thereof and 3' of the ATG start codon of the pre-C gene, wherein the fragment extends around the circular genome, across the junction of the first and last nucleotides of the genome, and terminates at a 3'

end corresponding to the nucleotide immediately preceding the "A" of the ATG start codon of the pre-C gene.

The "sub-genomic fragment" recited in claim 2 will be construed as a fragment of the circular HBV genome, wherein the 5' end of the fragment corresponds to the "A" of the ATG start codon of the pre-C gene, extends through "the +1 of transcription" as discussed above, and terminates at a 3' end corresponding to a nucleotide at or after the polyA attachment site.

The term "in 3' of" in claim 15 will be interpreted as "3' of".

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3, 4, 17, 37, 46, 47 and 60 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 17 and 60 recite the limitation "well conserved among HBV". This renders the scope of the claim indefinite since there is no explicit definition what is required to be considered "well conserved". Moreover, this language is relative, and reasonable people could disagree over the degree to which sequences must be conserved to be considered "well conserved".

Claims 17 and 60 are also objected to under this section based on the phrase "use is made of primers...". It is not clear what this limitation means. One interpretation could be that this refers to the amplifying in step (a) of claim 1. However, this could also

refer to any other "use" not recited in the claims, such as, for example, sequencing the amplification products, or "using" the primers to detect viral nucleic acid in the transfected cell cultures as a means of evaluating replication capacity. In *Ex parte Erlich*, a claim which read: "A process for using monoclonal antibodies of claim 4 to isolate and purify human fibroblast interferon." was held to be indefinite because it merely recites a use without any active, positive steps delimiting how this use is actually practiced. *Ex parte Erlich*, 3 USPQ2d 1011 (Bd. Pat. App. & Inter. 1986). Therefore, the metes and bounds of claims 17 and 60 are unclear, since it cannot be determined how the primers complementary to HBV genomic regions that are well conserved are used.

For purposes of examination over the prior art, it will be assumed the primers recited in claims 17 and 60 are used in the amplifying step (a) of claim 1.

Claim 37 recites the limitation "in the biological sample". There is insufficient antecedent basis for this limitation in the claim.

Claim 37 depends from claim 1, which has been amended to remove any reference to a biological sample. Therefore there is no longer any antecedent basis for the limitation recited in claim 37.

Claims 3, 4, 46 and 47 refer to GenBank AB048704. This renders the metes and bounds of the claims indefinite for the following reasons. Defining the scope of the claim in terms of a GenBank accession number is indefinite. GenBank sequences can be updated or modified, even though the accession number remains the same. This situation is described on pages 10-11 of the document entitled "Entrez Help", obtained from: http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/helpentrez/EntrezHelp.pdf on

05/11/2008. Specifically, the Entrez Help document states (page 11): "If a sequence changes in any way, it receives a new GI number, and the version number is incremented by one." Note that the GI number is not the same as the accession number, and the version number is the accession number followed by a dot and a version number (page 10). Hence it is possible for two different sequences to have the same accession number. Since, presently, there is only one version of GenBank accession number AB048704, Applicant is advised to recite the GenBank GI number, 13365548, since this number and the corresponding sequence are permanently linked.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 21 and 25-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

Evidence for the September 8th date for the Garces reference is provided in an email correspondence from Carol Wadke, supplied with the Office action mailed 08/20/2008.

With regard to claim 1, Junker taught a method of constructing a "greater-than-genome length" HBV construct (see figure 1) by cloning two fragments of HBV genomic DNA, which upon assembly represent a linear continuous DNA sequence "transcriptable in pgRNA", into a vector, thus producing a vector wherein transcription of pgRNA was under the control of a heterologous promoter (i.e. the human metallothionein II_A or "MT" promoter):

"First, the 2.3 kb BglII fragment 938-84 from pSHH2.1 was cloned into the BamHI site placing HBV position 938 near the HindIII site. Then, HBV sequences were completed by insertion of a HindIII-EcoRI fragment from plasmid pHTW3091 including HBV positions 3091-1280…The resulting plasmid was cut by SalI and HindIII and the MT promoter was inserted…" (paragraph bridging pages 10118-9, citations omitted).

"A slightly overlength HBV genome was cloned into the vector pUC13 between the unique HindIII and BamHI sites. This terminal redundant genome, starting 5' with the preC/C region and ending 3' with the HBV polyadenylation signal, contains all HBV genes in the order present in the pregenomic RNA. The human metallothionein IIA promoter was cloned in front of this genome. Thus, the constructs are expected to synthesize RNA molecules equivalent to the HBV pregenome" (page 10121, first paragraph).

Aside from the fact that Junker did not obtain the fragments used to make this construct by PCR amplification, this meets the limitations of steps (a) and (b) of claim 1.

Junker transfected susceptible cells with these vectors:

"Transfections were performed with 20 µg of DNA…" (page 10119, "Cells, immunoassays and protein analysis").

"In a first experiment, plasmid pMH3/3091 was introduced into HepG2 and HeLa cells…" (page 10121, "Transient expression in HeLa and HepG2 cells").

This meets the limitations of step (c) of claim 1.

In addition, Junker clearly stated: "Thus, the constructs are expected to synthesize RNA molecules equivalent to the HBV pregenome" (page 10121, first

paragraph). An RNA molecule equivalent to the HBV pregenome can be considered a pre-genomic RNA (pgRNA).

This meets the limitations of step (d) of claim 1.

Finally, Junker taught determining the replication capacity of the HBV (e.g. measuring the amounts of various viral products produced; see figure 2 for example).

This meets the limitations of step (e) of claim 1, since, as stated in Applicant's specification (paragraph [0128] of the published application), determining the replication capacity of the HBV "may involve measuring the level of nucleic acid synthesis, protein synthesis, and/or virus production".

With regard to claim 2, it is noted that the claimed "continuous DNA sequence" is indistinguishable from plasmid pMH3/3097 (see Junker figure 1). Junker describes in the first paragraph on page 10119: "Plasmid pMH3/3097 was derived from pMH3/3091 by replacing the HindIII-EcoRI HBV DNA fragment with the corresponding DNA fragment from pHTW3097 comprising HBV position 3097-1280 (9)." Reference 9 is the disclosure of Weimer et al.

Junker states on page 10121, second paragraph: "In addition, the preC start was eliminated in the third plasmid pMH3/3097 by deleting its first nucleotide...".

Thus, it is clear from the disclosure of Junker as evidenced by Weimer that position 3096 (using the numbering system of the Junker/Weimer references) corresponds to the "A" of the ATG start codon of the pre-C gene, while position 3097 corresponds to the "T". Therefore, the HBV sequence in Junker's pMH3/3097 began at the "T" of the ATG start codon of the pre-C gene and thus 5' of the "+1 of transcription"

(the transcription start site for the pre-genomic RNA). In addition, as can clearly be seen from Junker's figure 1, the HBV sequence extends to and includes the polyA addition site. Hence, aside from the fact that Junker did not obtain the fragments used to make this construct by PCR amplification, this meets the limitations of claim 2.

With regard to claim 3, the limitations describing "the continuous DNA sequence" in terms of position numbers of "an HBV genomic sequence aligned with" GenBank AB048704 do not distinguish over the HBV sequence in Junker's pMH3/3097 for the following reasons:

i. The term "comprising" allows any additional sequence, either at the ends or interspersed within the continuous DNA sequence, so long as the continuous DNA sequence contains in a 5' to 3' order the sequences within the recited position numbers.

ii. The recited position numbers do not refer to GenBank AB048704 itself, but to any HBV sequence *aligned* with the GenBank sequence; hence it need not be shown that Junker's plasmid comprised the GenBank sequence within the recited position numbers, but only sequence *corresponding* thereto.

iii. The examiner aligned nucleotides 1813 to 1960 of Norder figure 1 with those of GenBank AB048704 and found the sequences to be corresponding:

```
CATGCAACTTTTTCACCTCTGCCTAATCATCTCTTGTACATGTCCCACTGTTCAAGCCTC
||||||||||||||||||||||||||||||||||| ||| |||||||| ||||||||||||||
CATGCAACTTTTTCACCTCTGCCTAATCATCTCATGTTCATGTCCTACTGTTCAAGCCTC

CAAGCTGTGCCTTGGGTGGCTTTGGGGCATGGACATTGACCCTTATAAAGAATTTGGAGC
|||| |||||||||||||||||| || ||||||||||||||||||||||||||||||||||
CAAGTTGTGCCTTGGGTGGCTTTAGGACATGGACATTGACCCTTATAAAGAATTTGGAGC

TACTGTGGAGTTACTCTCGTTTTTGCCT
| |||||||||||||||| |||||||||
TTCTGTGGAGTTACTCTCTTTTTTGCCT
```

Hence, whatever aligns to the Norder sequence also aligns with the GenBank sequence in this region.

iv. The specification at page 7, last paragraph:

- an about 1-unit genome starting in 5' from and including the nucleotide representing the +1 of transcription (in general nucleotide A, position 1818 in H. Norder et al.; in some cases however, the +1 of transcription has revealed to be the nucleotide in 5' of said A, i.e. C, or in 3', i.e. A) to the first nucleotide in 5' of the ATG of the pre-C gene (nucleotide 1813 in H. Norder et al.), plus
- a sub-genomic fragment starting from and including the A of the ATG of the pre-C gene (nucleotide 1814 in H. Norder et al.) and extending to and including the polyA attachment site (nucleotide 1960 in H. Norder et al.), thus

Hence, Applicant identifies the "A" of the ATG start codon for the pre-C gene as position 1814 in the Norder sequence (which corresponds to position 1814 of the GenBank AB048704 sequence as well, i.e. the second nucleotide in the alignment shown above).

v. As discussed for claim 2 above, position 3097 (using Junker's numbering) corresponds to the "T" of the start codon of the pre-C gene, which in turn corresponds to position 1815 of the Norder figure 1 sequence, which in turn corresponds to position 1815 of the GenBank sequence.

Therefore, the continuous DNA sequence in Junker's pMH3/3097 begins with nucleotide 3097 (which corresponds to nucleotide 1815 of GenBank AB048704, according to the analysis discussed above), extends through the entire HBV genome, and ends the BglII site at nucleotide 84 (which corresponds to nucleotide 1984 of the GenBank AB048704, using the same analysis discussed above; see examiner's annotation on the GenBank printout). This being the case, Junker's continuous DNA sequence *comprised* from 5' to 3', nucleotides 1818 to 1813 and 1814 to 1960 (with an additional 3 nucleotides at the 5' end and an additional 24 nucleotides at the 3' end) of an HBV genomic sequence aligned with the sequence as set forth in GenBank AB048704. Aside from the fact that Junker did not obtain the fragments used to construct pMH3/3097 by PCR amplification, this meets the limitations of claim 3.

With regard to claim 25, Junker taught hepatoma cells (see Abstract: "Transient expression of this construct in hepatoma cells..."; see also page 10121, section entitled "Transient expression in HeLa and HepG2 cells").

With regard to claim 26, Junker directly transfected the cells with the vector (page 10119, first paragraph under "Cells, immunoassays and protein analysis": "Transfections were performed with 20 μg of DNA...").

Junker did not teach PCR amplifying HBV nucleic acids using at least two primer pairs selected so as to obtain at least two different fragments as recited in claim 1.

Junker did not teach any of the heterologous promoters recited in claim 21.

Junker did not teach transferring the vector into a baculovirus and then transducing the cells with the virus as recited in claim 27.

With regard to claims 1-3, Garces taught a method for constructing a greater-than-genome length HBV vector under control of a heterologous promoter (page 65). Specifically, a sub-genomic fragment beginning at the start codon of the core protein and extending to the unique HBV *Eco*RI site was amplified by PCR. This, along with a full-length genome fragment, were cloned under the control of the CMV promoter. See page 39 "wtHBV construct" for an illustration of the construct.

Garces did not explicitly teach that *both* fragments were obtained by PCR amplification; Garces is silent with respect as to how the full-length genomic fragment was obtained (see page 65: "...a linear full-length wild-type...genome was cloned to generate the greater-than-full length genome constructs".

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to synthesize the two fragments used by Junker to construct pMH3/3097 by PCR, thus arriving at the claimed invention, since the concept of using PCR to generate fragments to assemble into greater-than-genome length HBV sequences under control of a heterologous promoter was known as is clear from the disclosure of Garces. Although Garces only explicitly taught that one of the two fragments was made by PCR, one of ordinary skill in the art would have realized that if one fragment could be made by PCR, then the other fragment could have also been synthesized in this way as well.

The use of PCR for obtaining fragments, as taught by Garces, may be regarded as an improvement over the earlier method of Junker, which relied on the pre-existence of suitable restriction sites as well as numerous steps including elimination of restriction

sites, addition of linkers, subcloning and site-directed mutagenesis to introduce restriction sites, all of which would have required several days if not weeks to accomplish (see paragraph bridging pages 10118-9 of Junker). Therefore, in light of Garces, it would have been obvious to use PCR to obtain fragments since this could have been accomplished more rapidly, avoiding dependence on pre-existing restriction sites or the need to generate them through mutagenesis.

In addition, PCR would have allowed any desired fragments to be amplified from the HBV genome, as well as allowing the addition of restriction sites to the ends of the amplified fragments simply by designing primers with the restriction sites already included (see for example section 2.2 of Garces, beginning on page 30, where Garces describes amplifying regions of the HBV genome with primers comprising NheI restriction sites, digesting the amplified products with NheI and cloning the products into vectors also digested with NheI). Thus it would have been obvious to apply the improvements and advantages of PCR as exemplified by Garces to the method for constructing the greater-than-genome length HBV construct taught by Junker.

With regard to claim 21, Garces teaches a greater-than-genome length construct under control of the cytomegalovirus immediate early (CMV-IE) promoter (page 38, section 2.10, first sentence). It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention is made to substitute the CMV-IE promoter for the human metallothionein promoter in the construct of Junker, since both promoters were known in the art for controlling expression of HBV (see MPEP 2144.06).

With regard to claim 27, Garces transferred the construct into baculovirus which was then used to transduce (i.e. pseudo-infect) Hep G2 cells (page 66). It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to insert the heterologous promoter-driven greater-than-genome-length HBV sequences taught by Junker into a baculovirus for transduction of the cells. Garces taught that advantages of using baculovirus over transfection were simplicity and greater efficiency in terms of the ratio of cells expressing hepatitis B viral proteins (page 28).

Claim 18 is rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649
as applied to claims 1-3, 21 and 25-27 above and further in view of McLaughlin et al (US 2003/0104395).

The teachings of Junker and Garces have been discussed. These references did not teach or suggest any particular number of amplification cycles for amplifying fragments of HBV.

McLaughlin taught (paragraph [0060]): "PCR reaction time, temperatures and cycle numbers may be varied to optimize a particular reaction as a matter of routine experimentation."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to optimize the number of PCR cycles over the ranges recited in claim 18 as a matter of routine experimentation. As the court has stated, "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Claim 19 is rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:

http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

as applied to claims 1-3, 21 and 25-27 above and further in view of Pachuk et al (Gene 243:19-25 (2000)).

The teachings of Junker and Garces have been discussed. These references do not teach or suggest cloning the fragments into a vector using a one-step cloning procedure.

Pachuk taught a one-step cloning procedure (see entire article).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use a one-step cloning procedure to clone the amplified HBV fragments when constructing the vectors taught by Junker, because Pachuk taught his method "has made it possible to generate clones in one step that would require multiple steps by other methods" (page 25, last paragraph).

Claims 22-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:

http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

as applied to claims 1-3, 21 and 25-27 above and further in view of Wilson et al (USPN 6,001,557).

The teachings of Junker and Garces have been discussed. These references do not teach or suggest an actin promoter (as recited in claim 22), the chicken beta actin promoter (as recited in claim 23), or a beta actin associated with the CMV-IE enhancer (as recited in claim 24).

Wilson taught promoters for heterologous gene expression, including the CMV-IE promoter/enhancer and the CMV enhancer/chicken beta actin promoter (paragraph bridging columns 7-8). Furthermore, Wilson taught that the selection of such a promoter was a "routine matter" (column 7, line 65) and that "other promoter/enhancer sequences may be selected by one of skill in the art" (column 8, lines 10-12).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute the metallothionein promoter used by Junker with the CMV enhancer/chicken beta actin promoter taught by Wilson, as doing so would have been a "routine matter" and would have represented nothing more than the substitution of one promoter known to be useful for expression of heterologous genes with another such promoter (see MPEP 2144.06).

Claim 28 is rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649
as applied to claims 1-3, 21 and 25-27 above and further in view of Sells et al (Proc. Natl. Acad. Sci. USA 84:1005-1009, February 1987).

The teachings of Junker and Garces have been discussed. These references did not teach transferring the vector into a cell line in order to produce a stable cell line constitutively expressing HBV.

Sells taught transferring a plasmid bearing four tandem copies of the HBV genome into a cell line (HepG2) to produce a stable cell line constitutively expressing HBV (see Abstract: "HBV DNA is carried by these cells as chromosomally integrated sequences...", hence, stable).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to transfer the constructs made by Junker (using PCR to

generate the fragments as suggested by Garces) into a cell line for the purpose of producing a "stable" cell line constitutively expressing HBV, since Sells taught that such a system can "be used to study the life cycle of HBV and the reaction of immunocompetent cells with cells carrying HBV" (see Abstract).

Claims 38-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649
as applied to claims 1-3, 21 and 25-27 above and further in view of Delaney et al (Antimicrobial Agents and Chemotherapy 43(8):2017-2026, August 1999).

The teachings of Junker and Garces have been discussed. These references did not teach treating the cultured cells with a pharmaceutical product or a molecule (as recited in claim 38), an antiviral agent (as recited in claim 39), lamivudine (as recited in claim 40). These references also do not teach testing "potential antiviral agents" (as

recited in claim 41) or determining the "incidence" of the pharmaceutical agent on viral gene expression and/or replication (as recited in claim 42).

Delaney taught a method wherein a recombinant baculovirus expressing HBV was transduced to HepG2 cells (see Abstract and page 2018, last paragraph prior to section entitled "Materials and Methods"). In addition, Delaney treated the cultured cells with lamivudine (3TC) following the transduction (see, e.g., Abstract: "We have investigated the antiviral properties of 3TC in vitro in HepG2 cells infected with recombinant HBV baculovirus"; see also page 2018, "Materials and Methods", "3TC treatment": "In experiments in which 3TC treatment was initiated after viral infection, HepG2 cells were exposed to the indicated concentration of 3TC 24 h postinfection (p.i.) or 4 days p.i."). Moreover, Delaney stated that "the HBV baculovirus-HepG2 system has specific advantages for drug studies and can serve as a complement to other in vitro model systems currently used for testing antiviral compounds" (last paragraph, page 2025).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made apply the HBV construct of Junker (made by the more modern technique of PCR as suggested by Garces) to testing the effects of antiviral agents or potential antiviral agents, as suggested by Delaney. One would have been motivated to do this in order to derive new therapies for treating HBV infections.

Claim 20 is rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from:

http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:

http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

as applied to claims 1-3, 21 and 25-27 above and further in view of Junker-Niepmann et al (EMBO Journal 9(10):3389-96 (1990)) and Yadava et al (Molecular Biology Today 1(1):17-22 (2000)).

The teachings of Junker and Garces have been discussed. These references did not teach fusing the +1 of transcription of the heterologous promoter to the +1 of transcription of the HBV fragment.

Junker-Niepmann taught that the construct pMH3/3097 (the same construct taught in Junker et al (1987)), had 15 non-HBV nucleotides at the 5' end of the pre-genomic RNA which interfered with genomic circularization (page 3390, first section under "Results").

Yadava taught a solution for avoiding vector sequences in a transcript was to clone in such a way that no vector sequence was present in the transcript (i.e. joining

the +1 of transcription of the promoter in the vector with the +1 of transcription of the insert):

"We constructed two modifications of the Bluescript vector viz., pBgl II and pStuI, offering transcription initiation precisely at the insert (+1in pBgl II) or at two bases upstream of the insert (-2 in pStu I), from bacteriophage T7 promoter." (abstract)

"Vectors offering multiple cloning sites and the possibility of transcription of the insert usually result in transcripts with a chunk of vector sequences at the 5' end. It is essential to minimize these additional sequences to study the kinetics of ribozyme reactions and the influence if any of the associated sequences." (first sentence, "Introduction")

"Vectors described in this report offer sites for sticky as well as blunt end ligations that result in a G occupying +1 position for efficient transcription initiation and leaving minimal non-insert nucleotides in the transcripts." (sentence spanning pages 17-18)

"...sticky end ligation in BglII site places the insertsequence at +1 position leaving no vector nucleotides. The pBgl II vector can be adapted also for blunt end ligation after making flush ends either by S1 treatment or by filling the 5' overhangs with Klenow or T4 DNA polymerase reactions in the presence of all the nucleotides. If flush ends are created by S1 treatment, it will place the insert right at transcription initiation..." (passage spanning pages 20-21)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method suggested by the combined teachchings of Junker and Garces to place the +1 transcription of the HBV sequence at

the +1 transcription of the heterologous promoter to avoid the interference in packaging the pgRNA resulting from the vector sequence discussed by Junker-Niepmann.

Claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from:
http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.uml.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649
as applied to claims 1-3, 21 and 25-27 above and further in view of Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994).

The teachings of Junker and Garces have been discussed.

With regard to claim 45, see discussion of claim 2 above.

With regard to claim 46, see discussion of claim 3 above.

With regard to claim 64, see discussion of claim 21 above.

With regard to claim 68, see discussion of claim 25 above.

With regard to claim 69, see discussion of claim 26 above.

With regard to claim 70, see discussion of claim 27 above.

Junker and Garces did not teach the recited nucleotide position numbers recited in claims 4 and 47. Junker and Garces did not teach generating "overlapping" fragments by PCR, wherein the overlapping region comprised a restriction site, as recited in claims 5, 48 and 76, or the specific limitations concerning the primers and/or primer pairs recited in claims 10-15 and 53-58. Junker and Garces did not teach extracting the nucleic acids from a biological sample as recited in claim 37, or that the HBV is present in a biological sample as recited in claim 43, or that the identity of the HBV was "unknown" as recited in claim 44.

Hasegawa taught extracting HBV nucleic acid from a biological sample (first statement under "Materials and Methods", page 1651: "Serum DNA was purified from one of the patients with fulminant hepatitis B..."), thus meeting the limitations of claims 37 and 43. Hasegawa taught amplifying 3 overlapping fragments of said HBV nucleic acid covering the entire genome using 3 primer pairs (first section under "Materials and Methods" beginning on page 1651). Moreover, two of the fragments were joined together by using a restriction site (SpeI) found within the region of overlap (position 681), thus meeting the limitations of claims 5, 48 and 76. Hasegawa taught constructing a greater-than-genome length construct (page 1652, column 1, last full paragraph: "Terminally redundant genomes (1.2 times the genomic length) of two wild-type HBV strains were constructed...A similar construct of the fulminant HBV strain (FH) with 1.2 times the genomic length was generated." It is noted that the FH strain was

characterized by Hasegawa, thus was "unknown" when he extracted and amplified its DNA, thus meeting the limitations of claim 44.

With regard to claims 4 and 47, while Junker did not teach or suggest including the additional sequence downstream of the polyA site as indicated by the recited nucleotide position numbers in claims 4 and 47 (to arrive at the invention of claims 4 and 47, an additional 32 nucleotides would need to be added at the 3' end of the HBV sequence of Junker's pMH3/3097), Garces taught a greater-than-genome length construct extending from position 1818-3211-3212/1-3211 ("wildtype construct", page 65). Hasegawa taught a greater-than-genome length construct extending from 1417-3221/0-2184. Hence, it is clear that there was considerable latitude with regard to how to construct greater-than-genome length HBV replication-competent constructs. It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to simply add the next 32 nucleotides of the known HBV genomic sequence to the end of the construct taught by Junker, as this would have represented nothing more than combining prior art elements according to known methods to yield predictable results (MPEP 2141(III)(A)).

With regard to claims 5 and 48, it has been discussed above why it would have been obvious to use PCR to generate the fragments when constructing the vector taught by Junker. In doing so, one would necessarily have had to use a pair of primers for each fragment amplified, wherein each pair comprised a forward and a reverse primer. Furthermore, since Junker specifically constructed a vector containing a greater-than-genome length HBV sequence with the +1 of transcription at the 5' end

and the polyA site at the 3' end (see Junker figure 1, pMH3/3097) it would have been obvious that one fragment generated by PCR would have to comprise the +1 of transcription at the 5' end, while another fragment would have had to comprise the polyA site at the 3' end, such that upon assembling these fragments, the structure shown in Junker figure 1 would have resulted. Therefore it would also have been obvious that the forward primer of one primer pair would contain the nucleotide representing the +1 of transcription, since this was what was what was found at the 5' end of the HBV sequence in Junker's pMH3/3097.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to construct the HBV vectors taught by Junker by PCR (as suggested by Garces) by amplifying overlapping fragments wherein a restriction site found within the region of overlap was used to join the fragments (meeting the limitations in claims 5, 48 and 76). This was a known technique for assembling HBV genomic fragments as demonstrated by Hasegawa.

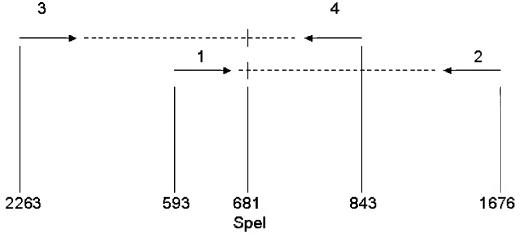
With regard to claims 10 and 53, it is noted that elected SEQ ID NO:13 was known as being found within the HBV genome as evidenced by GenBank AB048704:

```
Query  1372  CCATGGCTGCTAGGCTGTGCTGCC  1395
             ||||||||||||| |||||||||
Sbjct  24    CCATGGCTGCTAGGSTGTGCTGCC  1
```

(Note: "Query" is the GenBank sequence, whereas "Sbjct" is SEQ ID NO:13; "S" represents either C or G—see Specification page 12, next-to-last paragraph.) Thus, SEQ ID NO:13 would have been just as obvious a choice as any other known sequence

in the HBV genome for amplifying a first fragment of HBV comprising the +1 transcription region.

With regard to claims 11, 12, 54 and 55, it would also have been obvious that the "forward" primer of the second pair would have been 5' of the "reverse" primer of the first pair, with respect to the positions of the primers within the genome. Otherwise, there would be a gap left in the sequence. Hasegawa demonstrates this principle. Hasegawa's first primer set comprised a forward primer (primer 3) corresponding to nucleotide positions 2263-2287 and a reverse primer (primer 4) corresponding to nucleotide positions 823-843. Hasegawa's second primer set comprised a forward primer (primer 1) corresponding to nucleotide positions 593-617 and a reverse primer (primer 2) corresponding to nucleotide positions 1656-1676. These were used to amplify first and second fragments which were subsequently joined using the SpeI site found in the region of overlap (position 681; see Hasegawa page 1651, column 2, section entitled "HBV constructs" and examiner's illustration below):



As can be seen, the forward primer (1) of the second primer pair is 5' with respect to the reverse primer (4) of the first primer pair, and both primers are complementary to a region of HBV which comprises a natural restriction site (SpeI).

With regard to claims 13 and 56, it would have been obvious that any pair of overlapping fragments from the HBV genome could be amplified so long as they resulted, upon assembly, in the greater-than-genome length HBV sequence of Junker's pMH3/3097 or a functional obvious variation thereof. Thus, the choice as to where the fragments would overlap would have been arbitrary, and all options producing a functional construct would have been considered equivalent. Using the assembly technique of Hasegawa as a guide, one of skill would have seen that any region comprising a unique restriction site would have been a suitable region of overlap, and would have been able to design primers accordingly. Since, as evidenced by GenBank AB048704, the genome of HBV was known to contain a unique NcoI site (position 1372 of the GenBank sequence; see examiner's annotation on the GenBank printout), this, too, would have been an obvious place to design the overlap such that the fragments could be joined with that enzyme.

With regard to claims 14 and 57, it is noted that elected SEQ ID NO:15 was known as being found within the HBV genome as evidenced by GenBank AB048704:

```
Query  1363  ACCTCCTTTCCATGGCTGCTAGG  1385
             || || ||||||||||||||||||
Sbjct  1     ACMTCSTTTCCATGGCTGCTAGG  23
```

(Note: "Query" is the GenBank sequence, whereas "Sbjct" is SEQ ID NO:15; "S" represents either C or G—see Specification page 12, next-to-last paragraph; "M"

represents either A or C—see Specification page 13, third paragraph.) Thus, SEQ ID NO:15 would have been just as obvious a choice as any other known sequence in the HBV genome for amplifying a second fragment. In fact, the elected primers SEQ ID NO:13 (claims 10, 53) and 15 (claims 14, 57) would have been more obvious than other sequences from the HBV genome since they comprised a unique NcoI site of the HBV genome, and as discussed above, in light of Hasegawa's method of assembling fragments, making use of such a restriction site would have been standard technique.

With regard to claims 15 and 58, since Junker's pMH3/3097 comprised the polyA site at the 3' end (see Junker figure 1), it would have been obvious that a primer used to amplify a fragment comprising the polyA site would need to be located 3' of the polyA site in order to include the polyA site in the resulting PCR product.

With regard to claims 37, 43 and 44, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method suggested by the combined teachings of Junker and Garces by extracting and amplifying "unknown" HBV strains from patient samples and assessing their replication capacity, since this is precisely what Hasegawa did, demonstrating that it would have been of interest to one of skill in the art to do so.

Claims 6, 7, 49 and 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987)) as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:

http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of Jones (US 2002/0072055).

The teachings of Junker, Garces and Hasegawa have been discussed. In particular, Junker assembled a greater-than-genome length HBV sequence using fragments obtained by cutting them out of plasmids, whereas Garces introduced the idea of using PCR to obtain at least one of the fragments. Hasegawa taught using multiple primer pairs to obtain overlapping fragments with which to assemble an HBV genome.

Junker, Garces and Hasegawa did not explicitly teach a primer "partially complementary" to the 5' part of the pre-C gene, including the nucleotide represent the +1 of transcription, but not containing the ATG of the pre-C gene (as recited in claims 6 and 49) or comprising a restriction site that is not present in the HBV genome (as recited in claims 7 and 50).

Jones taught at paragraph [0044]: "Restriction endonuclease digestion is frequently used to generate cohesive ends for cloning DNA segments into a vector. This can be accomplished by attaching restriction endonuclease recognition domains to the ends of a DNA fragment by ligation of a linker or adaptor. Alternatively, a recognition domain can be incorporated into the end of a nucleic acid sequence using a primer whose 5' end contains the restriction endonuclease recognition site of interest, followed by primer directed synthesis of the opposite strand. One limitation inherent in such primer directed incorporation of a restriction endonuclease recognition domain is that the fragment of interest cannot contain the recognition domain for this enzyme if the intact fragment is to be cloned by digestion with this restriction endonuclease, as cutting of internal sites would generate shorter segments."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to incorporate restriction sites into the primers corresponding to the outer ends of the to-be-assembled greater-than-genome length sequence in order to clone the resulting sequence into the vector, and to use restriction enzyme sites that were not found in the HBV genome, in keeping with the suggestions of Jones. In this way, one would have arrived at the invention of claims 6, 7, 49 and 50, because this would have resulted in a forward primer that was only "partially complementary" to the 5' part of the pre-C gene. Furthermore, it would also have been obvious that this forward primer would contain the nucleotide representing the +1 of transcription (but not the ATG start codon of the pre-C gene), since this was what was what was found at the 5' end of the HBV sequence in Junker's pMH3/3097.

Claims 8 and 51 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))
as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from
http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649
and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994) and Jones (US 2002/0072055), as applied to claims 6, 7, 49 and 50 above, and further in view of Halle et al (USPN 6,303,308).

The teachings of Junker, Garces, Hasegawa and Jones have been discussed. These references did not teach or suggest the restriction enzymes recited in claims 8 and 51.

Halle demonstrates that all of these restriction enzymes were known in the prior art (column 3, lines 29-34).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use any of the enzymes recited in claimd 8 and 51 for as unique restriction sites on the primers for amplifying HBV fragments, since all these enzymes as well as the HBV genome sequence were known in the art. It would have been well within the skill of the ordinary artisan to determine which enzymes did not cut within the known HBV sequence, and the choice of any of the enzymes recited in claims 8 and 51 would have represented nothing more than the selection of a known material for its intended purpose (see MPEP 2144.07).

Claims 61, 77 and 78 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))
as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of McLaughlin et al (US 2003/0104395).

The teachings of Junker, Garces and Hasegawa have been discussed. These references did not teach or suggest any particular number of amplification cycles for amplifying fragments of HBV.

McLaughlin taught (paragraph [0060]): "PCR reaction time, temperatures and cycle numbers may be varied to optimize a particular reaction as a matter of routine experimentation."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to optimize the number of PCR cycles over the ranges recited in claims 61, 77 and 78 as a matter of routine experimentation. As the court has stated, "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Claim 62 is rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))
as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:

http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of Pachuk et al (Gene 243:19-25 (2000)).

The teachings of Junker and Garces have been discussed. These references do not teach or suggest cloning the fragments into a vector using a one-step cloning procedure.

Pachuk taught a one-step cloning procedure (see entire article).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use a one-step cloning procedure to clone the amplified HBV fragments when constructing the vectors taught by Junker, because Pachuk taught his method "has made it possible to generate clones in one step that would require multiple steps by other methods" (page 25, last paragraph).

Claims 63 is rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))

as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:

http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of Junker-Niepmann et al (EMBO Journal 9(10):3389-96 (1990)) and Yadava et al (Molecular Biology Today 1(1):17-22 (2000)).

The teachings of Junker, Garces and Hasegawa have been discussed. These references did not teach fusing the +1 of transcription of the heterologous promoter to the +1 of transcription of the HBV fragment.

Junker-Niepmann taught that the construct pMH3/3097 (the same construct taught in Junker et al (1987)), had 15 non-HBV nucleotides at the 5' end of the pre-

genomic RNA which interfered with genomic circularization (page 3390, first section under "Results").

Yadava taught a solution for avoiding vector sequences in a transcript was to clone in such a way that no vector sequence was present in the transcript (i.e. joining the +1 of transcription of the promoter in the vector with the +1 of transcription of the insert):

"We constructed two modifications of the Bluescript vector viz., pBgl II and pStuI, offering transcription initiation precisely at the insert (+1in pBgl II) or at two bases upstream of the insert (-2 in pStu I), from bacteriophage T7 promoter." (abstract)

"Vectors offering multiple cloning sites and the possibility of transcription of the insert usually result in transcripts with a chunk of vector sequences at the 5' end. It is essential to minimize these additional sequences to study the kinetics of ribozyme reactions and the influence if any of the associated sequences." (first sentence, "Introduction")

"Vectors described in this report offer sites for sticky as well as blunt end ligations that result in a G occupying +1 position for efficient transcription initiation and leaving minimal non-insert nucleotides in the transcripts." (sentence spanning pages 17-18)

"...sticky end ligation in BglII site places the insertsequence at +1 position leaving no vector nucleotides. The pBgl II vector can be adapted also for blunt end ligation after making flush ends either by S1 treatment or by filling the 5' overhangs with Klenow or T4 DNA polymerase reactions in the presence of all the nucleotides. If flush

ends are created by S1 treatment, it will place the insert right at transcription initiation..." (passage spanning pages 20-21)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method suggested by the combined teachchings of Junker, Garces and Hasegawa to place the +1 transcription of the HBV sequence at the +1 transcription of the heterologous promoter to avoid the interference in packaging the pgRNA resulting from the vector sequence discussed by Junker-Niepmann.

Claims 65-67 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))
as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649

and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of Wilson et al (USPN 6,001,557).

The teachings of Junker, Garces and Hasegawa have been discussed. These references do not teach or suggest an actin promoter (as recited in claim 65), the chicken beta actin promoter (as recited in claim 66), or a beta actin associated with the CMV-IE enhancer (as recited in claim 67).

Wilson taught promoters for heterologous gene expression, including the CMV-IE promoter/enhancer and the CMV enhancer/chicken beta actin promoter (paragraph bridging columns 7-8). Furthermore, Wilson taught that the selection of such a promoter was a "routine matter" (column 7, line 65) and that "other promoter/enhancer sequences may be selected by one of skill in the art" (column 8, lines 10-12).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute the metallothionein promoter used by Junker with the CMV enhancer/chicken beta actin promoter taught by Wilson, as doing so would have been a "routine matter" and would have represented nothing more than the substitution of one promoter known to be useful for expression of heterologous genes with another such promoter (see MPEP 2144.06).

Claim 71 is rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))
as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from: http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649 and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of Sells et al (Proc. Natl. Acad. Sci. USA 84:1005-1009, February 1987).

The teachings of Junker, Garces and Hasegawa have been discussed. These references did not teach transferring the vector into a cell line in order to produce a stable cell line constitutively expressing HBV.

Sells taught transferring a plasmid bearing four tandem copies of the HBV genome into a cell line (HepG2) to produce a stable cell line constitutively expressing HBV (see Abstract: "HBV DNA is carried by these cells as chromosomally integrated sequences…", hence, stable).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method suggested by the combined teachings of Junker, Garces and Hasegawa by transferring the constructs into a cell line

for the purpose of producing a "stable" cell line constitutively expressing HBV, since Sells taught that such a system can "be used to study the life cycle of HBV and the reaction of immunocompetent cells with cells carrying HBV" (see Abstract).

Claims 72-75 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))
as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649
and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of Delaney et al (Antimicrobial Agents and Chemotherapy 43(8):2017-2026, August 1999).

The teachings of Junker, Garces and Hasegawa have been discussed. These references did not teach treating the cultured cells with a pharmaceutical product or a

molecule, an antiviral agent, or lamivudine. These references also do not teach testing "potential antiviral agents".

Delaney taught a method wherein a recombinant baculovirus expressing HBV was transduced to HepG2 cells (see Abstract and page 2018, last paragraph prior to section entitled "Materials and Methods"). In addition, Delaney treated the cultured cells with lamivudine (3TC) following the transduction (see, e.g., Abstract: "We have investigated the antiviral properties of 3TC in vitro in HepG2 cells infected with recombinant HBV baculovirus"; see also page 2018, "Materials and Methods", "3TC treatment": "In experiments in which 3TC treatment was initiated after viral infection, HepG2 cells were exposed to the indicated concentration of 3TC 24 h postinfection (p.i.) or 4 days p.i."). Moreover, Delaney stated that "the HBV baculovirus-HepG2 system has specific advantages for drug studies and can serve as a complement to other in vitro model systems currently used for testing antiviral compounds" (last paragraph, page 2025).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method suggested by the combined teachings of Junker, Garces and Hasegawa by testing the effects of antiviral agents or potential antiviral agents, as suggested by Delaney. One would have been motivated to do this in order to derive new therapies for treating HBV infections.

Claims 17 and 60 are rejected under 35 U.S.C. 103(a) as being unpatentable over Junker et al (Nucleic Acids Research 15(24):10117-10132 (1987))

as evidenced by:

i. Weimer et al (Journal of Virology 61(10):3109-3113, October 1987)

ii. GenBank AB048704 [online] 23 March 2001 [retrieved on 6 August 2008] retrieved from http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=13365548

iii. Norder et al (Virology 198:489-503 (1994), cited on the IDS of 06/16/2006)

in view of Garces [online] publicly available on September 8, 2001 [retrieved on August 6, 2008], retrieved from:
http://proquest.umi.com/pqdweb?index=0&did=729292031&SrchMode=1&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1218309802&clientId=19649
and Hasegawa et al (Journal of Virology 68(3):1651-1659, March 1994), as applied to claims 4, 5, 10-15, 37, 43-48, 53-58, 64, 68-70 and 76 above and further in view of Liang et al (US 5,077,192).

The teachings of Junker, Garces and Hasegawa have been discussed. These references did not teach or suggest designing primers to "well conserved" regions of the HBV genome.

Liang taught (column 12, lines 35-45): "Other sequences of homology in the HBV genome have been examined and several oligonucleotide primers in other regions of interest such as in the pre-S and S domains have been selected. By comparison of available nucleotide sequences from different HBV subtypes (adw, adr, ayw) different sets of conserved DNA sequences to be used as primers can be defined. Each primer carries a restriction enzyme site at its 5' end for subsequent cloning."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method suggested by the combined teachings of Junker, Garces and Hasegawa by designing primers to conserved regions of the HBV genome as suggested by Liang. This would have offered the advantage of amplifying any HBV strain, which would have been particularly helpful when amplifying uncharacterized strains from patient samples as Hasegawa did.

Response to Arguments

Applicant's arguments filed 12/22/2008 have been fully considered but they are not persuasive. Applicant argues on page 18 of the response that the claimed methods allow for measuring the replication capacity of HBV present in a biological sample, and that by use of the methods claimed it is not necessary to know beforehand which particular strain of HBV is present in the sample. This is presumably because, as Applicant remarks, "[t]he primer pairs described and claimed in the present application are designed such that it is possible to recover nucleic acids from any or a vast number of HBV strains...". That is (as stated on page 19 of the reponse), "Applicants have unexpectedly discovered that primer pairs can be designed which are sufficiently universal such that by using the primers all of the necessary nucleic acids can be recovered from an HBV strain...".

With regard to the above rejections, these arguments are not persuasive because, other than claims 9, 10, 14, 16, 52, 53, 57 and 59, the claims are not limited to any particular primers. It is noted that claims 9, 16, 52 and 59 have been indicated free of the art with respect to the elected SEQ ID NOs 1 and 17. Furthermore, as regards

designing primers to conserved regions, as discussed in the rejection of claims 17 and 60 above, it was known in the art to do this (i.e. compare known related sequences, find those regions that are conserved, and design primers thereto in order to amplify/detect any of the related sequences). Therefore, this does not constitute an "unexpected" discovery, contrary to Applicant's assertion. Regarding the issue of amplifying HBV sequences from a "biological sample", as discussed above, this was also known in the prior art as disclosed by Hasegawa et al.

Conclusion

Claims 9, 16, 52 and 59 are free of the art with respect to the elected SEQ ID NOs 1 and 17.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/
Examiner, Art Unit 1637